The lactose permease meets Frankenstein.

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The lactose (lac) permease of *Escherichia coli* is a paradigm for membrane transport proteins. Encoded by the *lacY* gene, the permease has been solubilized, purified to homogeneity, reconstituted into phospholipid vesicles and shown to catalyze the coupled translocation of β-galactosides and H^+ with a stoichiometry of unity. Circular dichroism and other spectroscopic approaches demonstrate that purified permease is about 80% helical. Based on hydropathy analysis of the primary amino-acid sequence, a secondary structure has been proposed in which the protein has 12 hydrophobic domains in α-helical conformation that traverse the membrane in zig-zag fashion connected by hydrophilic loops. A variety of other approaches are consistent with the model and demonstrate that both the N and C termini are on the inner surface of the membrane, and studies on an extensive series of lac permease-alkaline phosphatase fusion proteins provide exclusive support for the topological predictions of the 12-helix motif. This presentation will concentrate on the use of molecular biological techniques to study structure-function relationships in the permease.